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Comprehensive Enhancement of Mechanical, Water-Repellent and Antimicrobial Properties of Regenerated Seaweed and Plant-Based Paper with Chitosan Coating

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Abstract: Regenerated papers made from discarded natural sources, such as seaweeds or non-wood plants, are viewed as promising eco-friendly alternatives relative to conventional wood-based paper. However, due to its limited mechanical strength and higher water absorption than compared to traditional wood paper, it often results in premature structural disintegration. In order to overcome this limitation, this research introduces an efficient and comprehensive strategy of coating seaweed and plant papers with varying concentrations and molecular weights of chitosan. Increased concentration and molecular weight resulted in a greater amount of chitosan deposition, while the highest molecular weight also shows increased dissolution of soluble components of the paper. Since plants and seaweeds contain high anionic polysaccharide contents, the cationic chitosan shows high binding affinity towards paper. The resulting chitosan-coated papers demonstrate significant enhancements in water repellency and mechanical properties. In addition, the chitosan-coated papers also show significant bacterial inhibition effects due to the natural anti-microbial activity of chitosan.

Keywords: regenerated paper; seaweed; non-wood plant; chitosan; coating; water repellency; mechanical properties; antimicrobial effect

1. Introduction

Deforestation is one of the most critical issues impacting the world, as it results in irreparable damage to the ecosystem; loss of habitat for countless species; increase in carbon emission; erratic climate change; and spread of infectious diseases [1–3]. In addition to land development for industry and human habitat, acquiring wood pulp for paper production has been a major contributor of deforestation. Despite the recent global efforts in recycling as well as the rise in portable electronics such as laptops, tablets and smartphones, which have all contributed to decreases in paper usage in printing applications, overall global paper production continues to remain high. Furthermore, environmental pollution arising from paper waste is a critical threat to our health.

In order to reduce the consumption of paper products and their waste, pulp made from seaweed biomass is increasingly viewed as a promising eco-friendly alternative to the traditional wood pulp-based papers [4–7]. Most seaweeds that are naturally overgrown along near-coastal waters, often called 'algal bloom', are viewed especially harmful to marine ecology, as it results in decreased oxygen levels to the detriment of marine life [8]. In addition, certain types of seaweeds produced in ocean farms are used for extracting mostly food and medical ingredients, leaving a large amount of seaweed waste. As a result, significant efforts are invested worldwide in order to remove seaweed waste. Therefore, taking advantage of seaweed waste to generate pulp can not only aid in cleaning up the ocean but also reduce deforestation. Furthermore, seaweed pulp more readily dissolves and decomposes in landfills or water by microbial activities than fibrous wood pulp. For these reasons, repurposing seaweed biomass has been increasingly adopted in several industrial applications, most notably for the production of various chemicals by using biorefinery and absorbent material for wastewater clean-up [9,10].

Another natural source that could significantly enhance the eco-friendliness of pulp production include agricultural plant byproducts [11–13]. After harvesting and industrial processing for obtaining food and drug products, exorbitant plant byproducts are generated and discarded. Only some of them are repurposed into animal feed or fertilizers. Even though they are not as mechanically strong as wood fibers, the plant byproducts do contain lignocellulosic fibers. Therefore, they possess a similar potential as the pulp material for paper production [12,13]. Furthermore, similarly to seaweed pulp, plant pulp more readily dissolves and decomposes underground or in water by microbial activities than compared to wood pulp due to higher concentrations of more readily degradable components, such as hemicellulose, pectin and ash [14–16].

Despite the environmental advantages of seaweed and non-wood plant biomass, their lack of mechanical strength and the resulting susceptibility to premature disintegration, especially in the presence of water, have been huge deterrents to more widespread utilization. Wood pulp mostly contains lignocellulosic fibers that naturally possess high mechanical strength [17]. The ranges of lignin contents for wood and non-wood plants have been determined to be 25%–35% and 5%–20%, respectively [18]. On the other hand, red and brown algae, the main ingredients of seaweed pulp, consist of non-fibrous polysaccharides, such as alginate, carrageenan and fucoidan, which are mechanically weaker and show a much greater rate of dissolution under water [19–21]. Soluble polysaccharides in red and brown seaweed generally range from 30% up to 40%, while being mostly devoid of lignocellulosic fibers [22]. Non-wood plants generally have shorter lignocellulosic fibers and contain greater hemicellulose and pectin contents that are more aqueous soluble than wood fibers [16]. For example, the ranges of hemicellulose contents for wood and non-wood plants usually are 20%–35% and 30%–40%, respectively. More remarkably, there is a greater difference in pectin content: up to 5% for wood and up to 35% for non-wood plants [23]. However, compared to non-fibrous seaweed, it is easier to generate more durable paper by using non-wood plant pulp possessing lignocellulosic fibers, even without any wood pulp.

In order to overcome this issue and to help increase the practical usability of seaweed and plant-based papers, this study presents a simple, cost-effective and yet highly efficient method of coating the papers with chitosan, which has not been explored in eco-friendly papers to our knowledge (Figure 1). Chitosan is a natural cationic polysaccharide derived via deacetylation of chitin, which is mostly found in exoskeletons of crustaceans [24,25]. Due to the abundance of amine groups, one in each saccharide unit, chitosan demonstrates severely limited solubility towards neutral and basic aqueous solutions and demonstrates natural antimicrobial effects. With these attributes, chitosan is actively investigated as a coating material for food and drug preservation [26–29]. In a similar fashion, it was hypothesized that the presence of chitosan would promote water-repellent and antimicrobial properties of seaweed and plant papers. In particular, since most seaweeds used for pulp production, such as brown and red algae, contain anionic polysaccharides, such as alginate, carrageenan and fucoidan, and non-wood plants also contain high concentrations of anionic hemicellulose and pectin, it was expected that cationic chitosan would demonstrate enhanced binding affinity towards seaweed and plant papers, resulting in mechanical reinforcement to prevent their premature structural disintegration (Figure 2). The concentration and molecular weight of chitosan were controlled in order to find the optimal coating conditions that would provide maximal increases in water repellency and mechanical strength of chitosan-coated papers. Furthermore, the ability of chitosan to suppress bacterial growth on chitosan-coated paper was also evaluated.

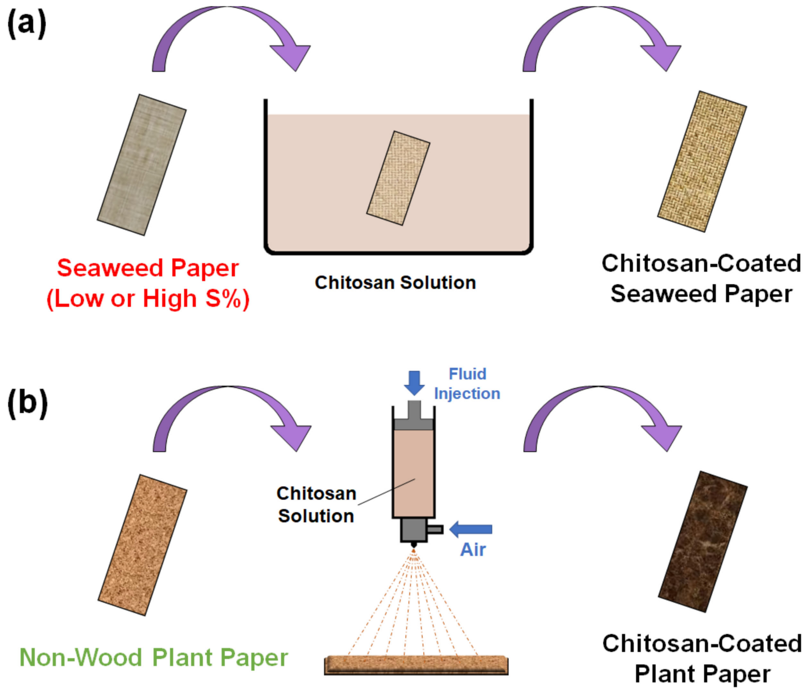

Figure 1. Chitosan was coated on (**a**) seaweed paper by dip coating or (**b**) plant paper by spray coating.

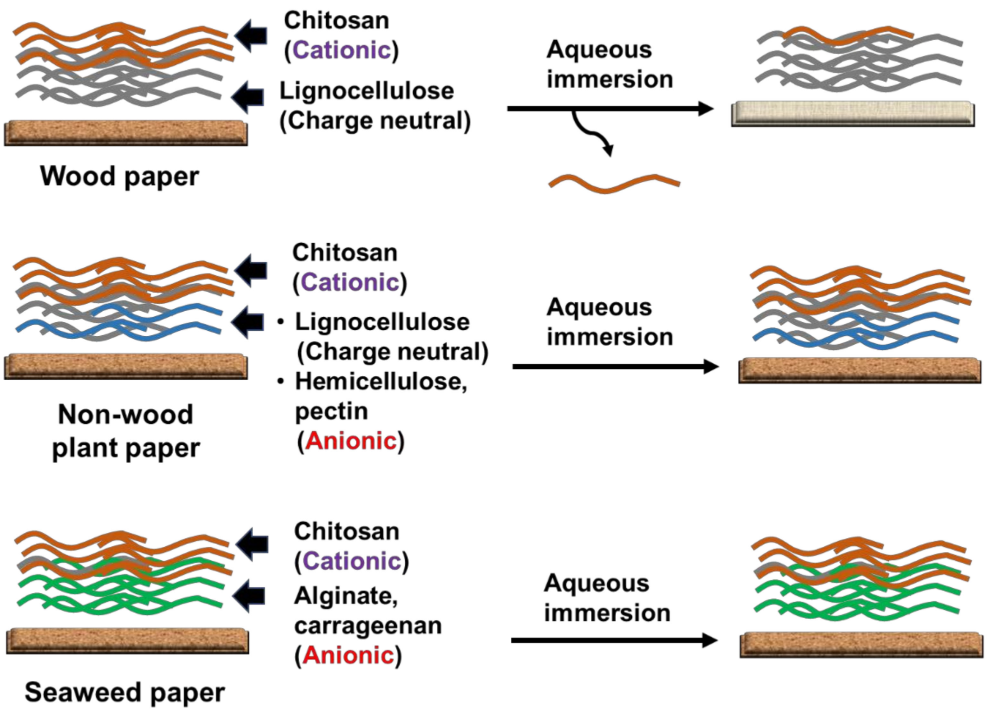

Figure 2. Schematic illustration of wood, non-wood plant and seaweed papers coated with chitosan with different binding affinities. Non-wood plant and seaweed papers having anionic components demonstrate higher binding affinities than wood paper, which is charge neutral.

2. Materials and Methods

2.1. Chitosan Coating on Paper

Chitosan possessing various molecular weights were dissolved in 2% acetic acid at low M_W (50,000–190,000 Da), medium M_W (200,000–300,000 Da) and high M_W (310,000–375,000 Da), all purchased from Sigma Aldrich, MO, USA. Their concentrations were controlled at 0.1%, 0.25% and 0.5%. Two different seaweed-based papers based on seaweed content, 8 wt.% ("Low S%") and 30 wt.% ("High S%") relative to wood pulp, were used (Marineinnovation Co., Korea, https://www.marineinv.com). Paper made from non-wood plant pulp, consisting of rice straws, corn stalks, reed and bagasse, was also used (Ecostech Co., Korea, http://www.ecostech.co.kr/). For seaweed papers, a dip coating method was used (Figure 1a) [30,31]. Paper samples were immersed into a chitosan solution for 1 min. After taking the samples out and removing excess solution, they were dried in vacuo at 60 °C. The amount of chitosan coated onto the paper was assessed by measuring the weight difference before and after the chitosan coating. The presence of chitosan on paper samples was confirmed with FT-IR spectroscopy (Bruker Alpha, Berlin, Germany) and a TNBS (2,4,6-Trinitrobenzene sulfonic acid) assay that quantified the amount of amine in chitosan (Figure S1) [32,33]. Scanning electron microscopy (SEM) was employed to visualize the detailed morphology of pulp structure (Model S-4800, Hitachi, Tokyo, Japan). Fiber thickness was measured from the SEM images; 20 fibers were measured, and their average and standard deviation were reported. The surface topographical features were visualized by using optical coherence tomography (OCT). Detailed schematics and working principles of OCT technology are provided in detail elsewhere [33].

For plant paper, a spray coating method was used. The spray coater (Model SRC-100, E-FLEX, Bucheon, Korea) consisted of an atomizing nozzle connected with fluid and air injectors, mounted on a lateral-moving stage (Figure S2). The flow rate of the chitosan solution was 10 mL min^{-1}, and the air pressure was 0.2 MPa. The nozzle diameter was 0.4 mm for 0.1% chitosan and 1.7 mm for 0.25% and 0.5% chitosan. The spray nozzle was scanned over the entire area of the sample paper during spraying. The process was repeated twice and dried in vacuo at 60 °C.

2.2. Mechanical Characterization of Chitosan-Coated Paper

2.2.1. Water-Repellent Effect

The chitosan-coated paper samples were immersed in hot water (at 80 °C) for 5 min. The weight of water absorbed paper (Q_W) was measured and compared with that of the dried paper (Q_D) in order to evaluate the water-repellent properties of chitosan coating. Water absorption capacity (WAC) was calculated with the following equation [34].

$$\mathrm{WAC}\ (\%) = \frac{Q_W - Q_D}{Q_D} \times 100 \quad (1)$$

2.2.2. Mechanical Properties

Tensile mechanical properties of chitosan-coated paper were evaluated using a universal testing machine (Model 3343, Instron, MA, USA). Briefly, each paper sample (2 cm × 6 cm) with or without chitosan coating was slowly stretched at 10 mm min^{-1}, and the stress–strain curve was obtained. The tensile modulus was calculated as the slope at the initial 10% strain, and fracture strength was chosen the highest stress value [33].

2.2.3. Contact Angle

The amount of 30 µL of a water droplet was placed on a paper sample. After 10 s, a picture showing the lateral view of the droplet was taken. The angle between the water-air interface and the paper surface was measured [35]. The average and standard deviation from 5 independent experiments were reported.

2.3. Antimicrobial Characterizations of Chitosan-Coated Paper

A standard bacterial culture protocol was followed to generate *E. coli* solution [33]. Briefly, 1 mL of frozen *E. coli* stock was thawed and transferred to fresh 9 mL Luria–Bertani (LB) media and cultured in a shaking incubator (37 °C, 150 rpm) overnight.

Each circular paper sample (0.5 cm diameter) was gently swabbed with *E. coli* solution and then immersed in 3 mL fresh LB media overnight. The bacterial growth in the media was measured with colorimetric MTT assay [36,37]. Briefly, 100 μL of media was taken and mixed with 10 μL MTT solution (5 mg mL^{-1} 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and incubated for 4 h at 37 °C. The amount of 100 μL of MTT stop solution was added and further incubated overnight at room temperature. The absorbance at 570 nm of the solution was measured (Multiskan GO, Thermo Fisher, Waltham, MA, USA). The degree of inhibition (%) for each sample was obtained by comparing with the negative control (pure medium) and positive control (uncoated sample).

3. Results and Discussion

3.1. Chitosan Coating on Seaweed and Plant Papers

In order to achieve greater extent of eco-friendliness of the seaweed paper, it is desirable to increase the seaweed content of the paper in order to expedite biodegradation and to reduce the consumption of wood pulp. However, seaweed pulp that mostly contains non-fibrous components cannot form paper that is strong enough by itself. The seaweed pulp used in this study was mostly brown algae, which contained high concentrations of anionic polysaccharides such as alginate and fucoidan, up to 56%, which are structurally weaker than lignocellulosic fibers [38]. Seaweed also contains significant amounts of minerals up to 40%, called ash, which can be readily dissolved [39]. Thus, seaweed pulp and wood pulp were mixed to generate the seaweed-based papers. Here, seaweed papers with two different concentrations of seaweed pulp were used, 8 and 30 wt.%, denoted as 'Low S%' or 'High S%' seaweed paper, respectively. Increasing seaweed content resulted in an overall increase in darkened hue. The greater presence of dark seaweed contents having various sizes indicated the inhomogeneous nature of seaweed pulp. In addition, increasing seaweed content resulted in increased surface coarseness due to the inhomogeneous nature of seaweed pulp preventing cohesive and compact paper formation with wood pulp (Figure 3). Detailed morphology as visualized by SEM revealed a larger presence of thinner seaweed fibers with increasing seaweed content, with average fiber thicknesses of 35 (±7) and 22 (±6) μm for Low S% and High S% seaweed papers, respectively

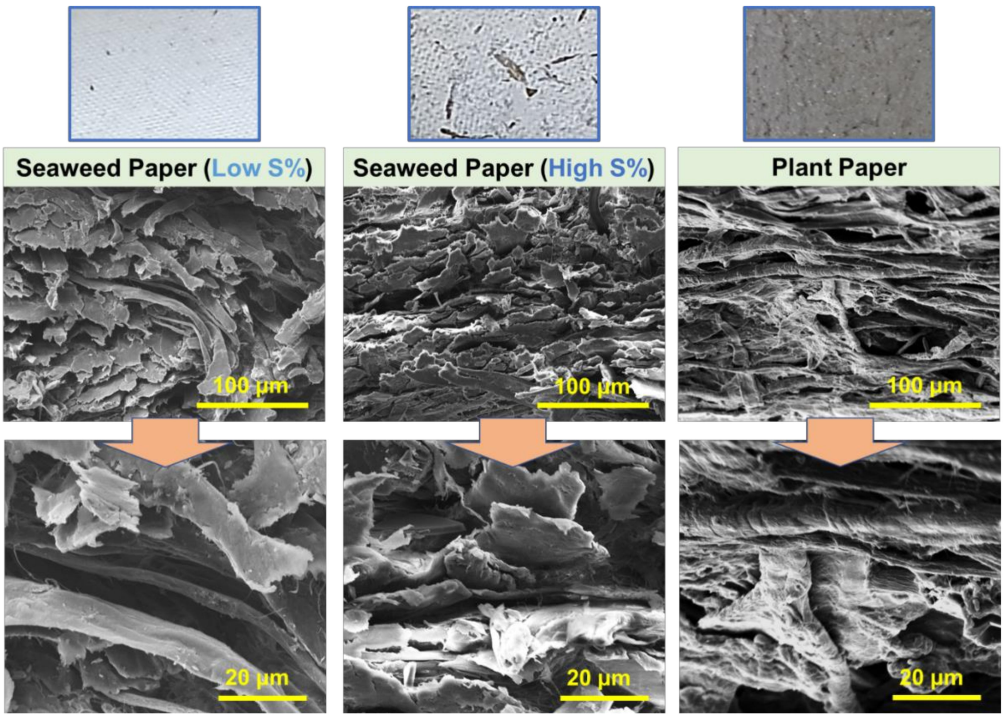

Figure 3. Photographs and SEM images of seaweed papers with different seaweed pulp contents (Low and High S%) and plant paper.

Plant paper, on the other hand, was made solely from non-wood plant pulp. The plant pulp consisted of higher concentration of pectin (30%–35%) and hemicellulose than wood pulp. Plant paper, devoid of wood pulp altogether, showed overall dark colors. The coarse surface texture was highly noticeable to a much greater extent than seaweed papers. SEM image also revealed that it is made up of thinner fibers than seaweed papers that contain wood pulp, with the average fiber thickness of 16 ($\pm$4) µm. These observations all indicated relative structural weakness of non-wood plant paper to seaweed papers that still mostly contain wood pulp.

In order to measure the efficiency of chitosan coating on seaweed paper, the amount of chitosan being deposited was measured. The effect of molecular weight (M_W) of chitosan was explored by using three different M_W's: low M_W ('L-Cs'), medium M_W ('M-Cs') and high M_W ('H-Cs'). The concentration of chitosan was controlled up to 0.5%, above which it resulted in critically high viscosity that prevented efficient absorption through the paper. FT-IR spectroscopy and TNBS assay all confirmed the presence of chitosan on the paper (Figures S1 and S3). To more quantitively analyze the chitosan coating, the change in overall weight of the paper after chitosan coating was measured.

Coating methodologies were selected based on different production values of seaweed paper and non-wood plant paper. For seaweed paper, seaweed pulp was only partially included in conventional wood paper, as seaweed itself does not form durable paper. The resulting seaweed-based paper is much closer to wood paper. Therefore, dip coating was employed here, as it is more cost-effective and more adequate for larger scale and traditional paper applications. It also allows more extensive deposition of coating material into the substrate while causing minimal structural damage.

For non-wood plant pulp, it can form paper by itself without the presence of wood pulp. Devoid of wood pulp, it is mechanically weaker but more readily biodegradable than wood paper, thereby rendering it more eco-friendly. It is largely restricted for temporary and smaller-scale usage due to their weak mechanical strength and facile aqueous dissolution. Spray coating was employed in this case, as this type of paper is not suitable for dip coating which result in significant structural damage. Spray coating also has the advantage of precisely controlling the amount of coating material being deposited.

3.1.1. Dip Coating-Seaweed Paper

The dip coating of paper samples was performed up to three times in order to control the amount of chitosan being deposited. Interestingly, the weight of Low S% seaweed paper decreased after the first coating regardless of chitosan, which indicated that small amounts of soluble components including non-fibrous polysaccharides, hemicellulose and pectin, smaller fibers, ash and additives contained in the paper were washed out (Figure 4a). Similar reductions in weight were also observed for High S% seaweed paper at 0.1% chitosan, but the weight did increase at 0.25% and 0.5% chitosan. This suggested that chitosan was more effectively incorporated into the seaweed paper with higher seaweed content. The effect of molecular weight of chitosan was also apparent, where the decrease in weight was noticeably larger at H-Cs. Due to the greater viscosity and ability to undergo more extensive physical interaction by higher M_W chitosan, it was speculated that soluble components became more easily leached out. Most soluble polysaccharides, hemicellulose and pectin are anionic; thus, excess cationic chitosan could facilitate the dissolution into chitosan solution via electrostatic interaction.

After subsequent coating iterations, the total weight gradually increased for Low S% paper, which indicated successful chitosan deposition (Figure 4b,c). It also showed that the dissolution of soluble components was negligible or at least not as significant as the weight gain by chitosan. However, there was a greater weight loss after the second coating for High S% seaweed paper, which demonstrated the continued dissolution of soluble components of seaweed pulp was still a significant factor (Figure 3e). Nevertheless, the weight of High S% seaweed paper did demonstrate significant increases after the third coating, even higher than Low S% seaweed papers (Figure 4f). Considering the greater tendency for aqueous dissolution, this result strongly indicated that a greater amount of chitosan could be deposited onto seaweed paper with higher seaweed content, far outweighing the dissolution of soluble components. Since seaweed contains anionic polysaccharides, such as alginate and fucoidan, cationic chitosan could be more effectively incorporated into the seaweed paper via electrostatic interaction [19–21]. This explanation was further supported by the fact that the weight continued to increase with increasing chitosan concentration up to 0.5% for High S% seaweed paper, but weight increase was the highest at 0.25% and became significantly diminished at higher 0.5% for Low S% paper.

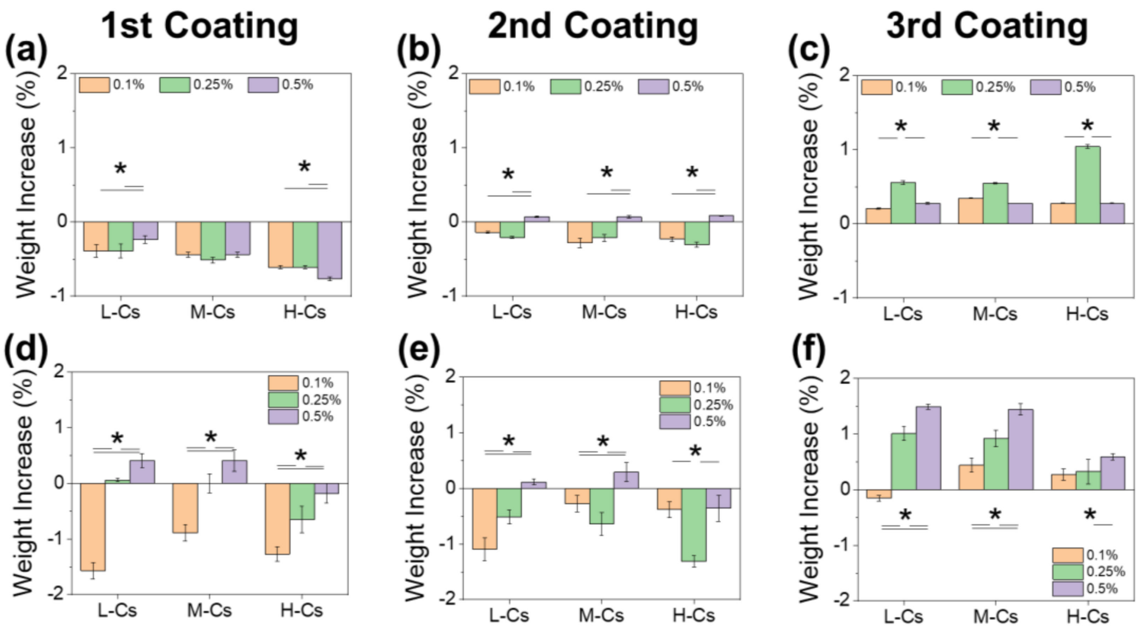

Figure 4. The increase in weight of paper samples after dip coating with chitosan: (**a**–**c**) Low S% seaweed paper and (**d**–**f**) High S% seaweed paper. The coating was performed up to three iterations (* $p < 0.05$).

It is also noteworthy to point out the varying effects of M_W of chitosan on the coating efficiency of seaweed papers. After the third coating, weight increase was the largest at the highest M_W of chitosan, H-Cs, for Low S% seaweed paper. On the other hand, weight increase was the lowest at H-Cs for High S% seaweed paper. This completely opposite effect could be attributed to the nature of chitosan incorporation to the paper. With Low S% seaweed, the chitosan was mostly incorporated via non-specific physical interaction (i.e., van der Waals force and chain entanglement) to wood pulp, where having longer chain lengths would increase the degree of interaction. On the other hand, with High S% seaweed paper in which greater chitosan incorporation is mediated by electrostatic interaction to the seaweed pulp, it may have been difficult for longer chitosan chains having higher chain stiffness to undergo electrostatic interaction than compared with shorter chitosan chains that could more effectively infiltrate the pulp. This effect was likely in combination with the greater loss of soluble components of seaweed pulp by higher M_W chitosan.

3.1.2. Spray Coating-Non-Wood Plant Paper

Unlike seaweed paper, plant paper devoid of wood pulp quickly lost their structural integrity after dip coating. Therefore, spray coating was employed instead to deposit chitosan without significant dissolution of plant pulp. The increase in total weight after chitosan coating was more pronounced than compared with seaweed paper and expectedly increased with chitosan concentrations (Figure 5). Since plant paper consists of shorter and less cohesive lignocellulosic fibers and anionic hemicellulose and pectin, chitosan solution could infiltrate and bind to the plant paper more effectively. It was also apparent in the characteristic brown hue of chitosan. However, the increase in weight with concentration was significantly lower at H-Cs, which was similarly demonstrated for Low S% seaweed paper, and decreased with concentration. Increasing the molecular weight of chitosan from L-Cs to M-Cs results in a small increase in weight; compared to L-Cs and M-Cs, the weight increase was significantly lower for H-Cs and surprisingly decreased with concentration. This suggested that, similarly to dip coating of seaweed paper, higher M_W chitosan likely induced substantial structural disintegration through electrostatic interaction by partially dissolving plant pulp. Nevertheless, spray coating a small amount of chitosan solution, instead of full immersion in dip coating, helped deposit chitosan effectively onto plant paper.

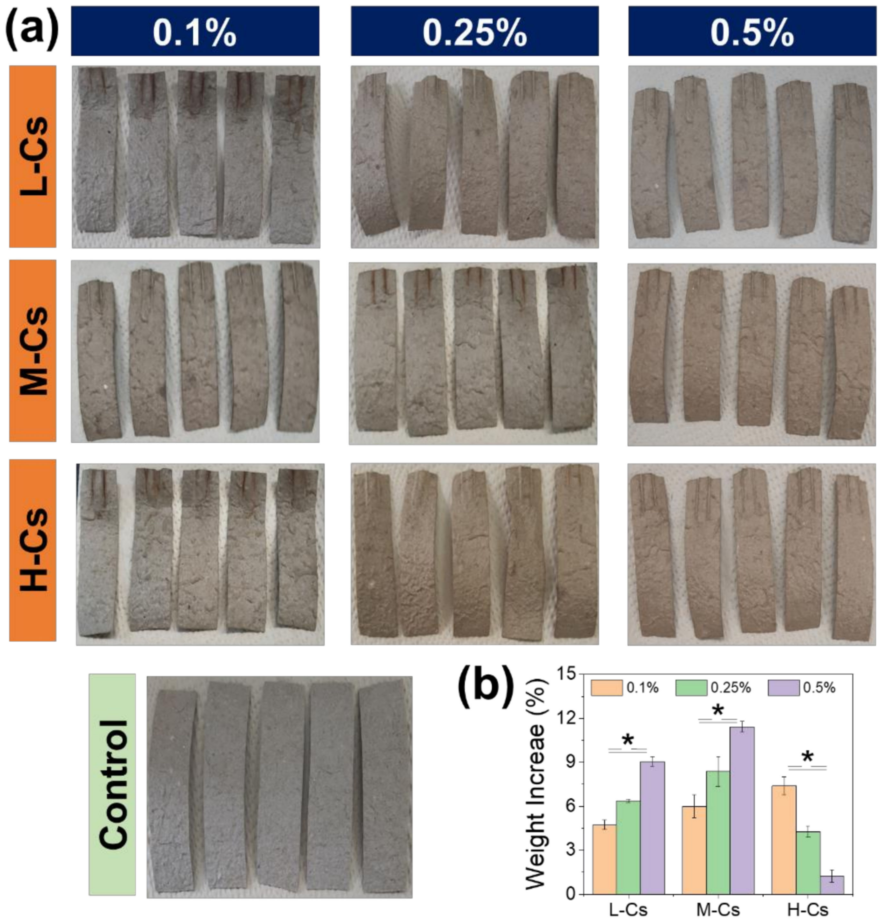

Figure 5. (**a**) Photographs of plant papers coated with chitosan with varying concentrations and molecular weights. (**b**) The increase in weight of plant papers after chitosan coating (* $p < 0.05$).

3.2. Water Repellent Properties of Chitosan-Coated Paper

Due to its cationic nature, chitosan does not dissolve well in neutral or basic conditions, demonstrating solubility only in acidic conditions. Therefore, chitosan has been widely used as a protective coating against moisture for packaging and storage [26,27]. This property of chitosan was expected to provide the same benefit for seaweed and plant papers, which are especially vulnerable to moisture. To demonstrate the water repellent effect, water absorption capacity (WAC) of chitosan-coated papers, after immersion in hot water, was evaluated.

For High S% seaweed paper, 0.1% chitosan coating did not provide any water repellent effect. On the contrary, there was a small increase in WAC than compared with that of the uncoated control at 61% (Figure 6a,b). This surprising result highlighted that the amount of deposited chitosan was not significant enough to impart water repellent effects, while a structural damage from leached seaweed pulp by chitosan likely facilitated water uptake. When the concentration was increased to 0.25%, chitosan coating showed much improved water repellent effect, as WAC decreased to 43% with L-Cs. However, WAC increased to 60% and 64% with increasing M_W of chitosan, M-Cs and H-Cs, respectively. This result similarly suggested that increased loss of seaweed pulp at higher chitosan concentration and M_W was significant enough to offset the water repellent effect by chitosan. When chitosan concentrations further increased to 0.5%, excellent water repellent effect was demonstrated, with WAC values ranging from 31% to 35% regardless of M_W of chitosan. This result clearly demonstrated that further increase in chitosan coating was substantial enough to overcome the small structural damage caused by chitosan, resulting in greatly improved water repellent effects.

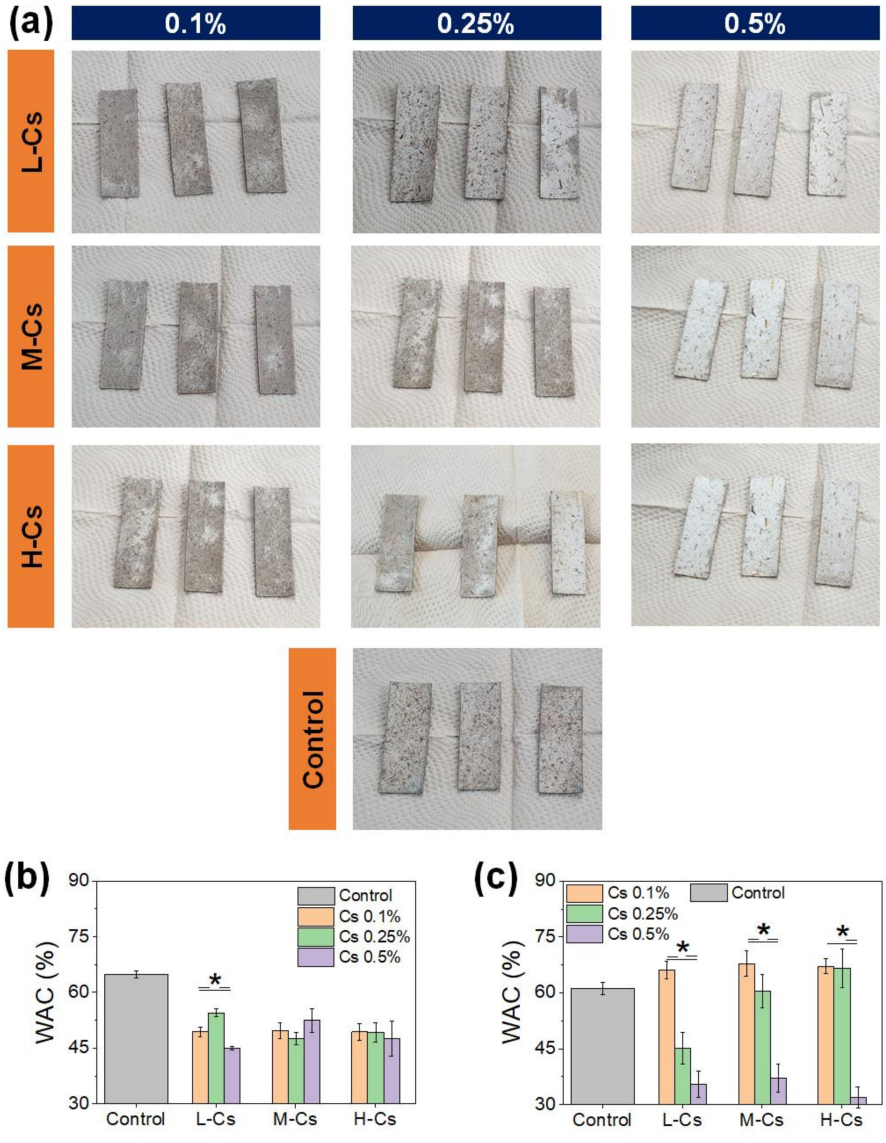

Figure 6. (**a**) Photographs of seaweed paper samples (High S%) coated with chitosan with varying concentrations and molecular weights, after immersing them in hot water. Water absorption capacity (WAC) values of (**b**) High S% and (**c**) Low S% seaweed papers coated with chitosan (* $p < 0.05$). Uncoated papers were used as control.

For Low S% seaweed paper, there was also a marked decrease in water absorption after chitosan coating, as WAC became as low as 45%, which was lower than the uncoated paper of 65% (Figure 5b, Figure S4). Similarly to High S% seaweed paper, chitosan coating on Low S% seaweed paper could also impart water repellent effect. However, there was no clear difference in WAC among chitosan with varying concentrations nor M_W. It is likely that chitosan coated on Low S% seaweed paper was not as strongly attached to High S% seaweed paper, and some of the chitosan may have been detached under aqueous conditions at elevated temperatures.

The water repellent effect was further evaluated at plant paper (Figure 7). Due to its inherent structural weakness, low fiber dimensions and density and high susceptibility for water absorbency, the uncoated paper became easily wet, with the WAC value as high as 320%. On the other hand, the WAC of chitosan-coated plant papers decreased substantially below 210% for 0.1% L-Cs and as low as 170% for 0.5% H-Cs. In addition, WAC gradually decreased with increasing chitosan concentration and M_W. This result clearly demonstrated the efficacy of chitosan coating on imparting water repellency even for seaweed paper with critically high susceptibility for water absorption by strongly binding to the seaweed pulp via electrostatic interaction.

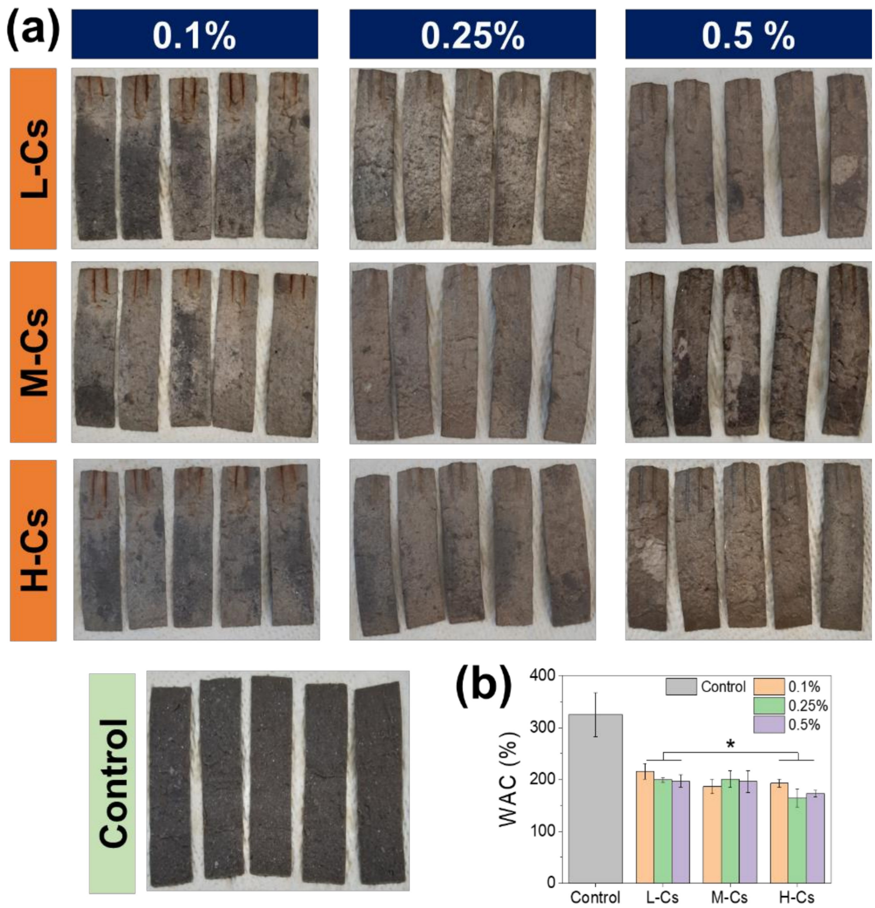

Figure 7. (**a**) Photographs of plant paper samples coated with chitosan with varying concentrations and molecular weights after immersing them in hot water. (**b**) Water absorption capacity (WAC) values of plant papers coated with chitosan (* $p < 0.05$). Uncoated paper was used as control.

3.3. Mechanical Properties of Chitosan-Coated Paper

The effect of chitosan coating on improving the mechanical strength of the papers was also assessed by measuring tensile moduli and fracture strengths (Figure 8, Figure S5). It was first hypothesized that chitosan coating would act as a reinforcing element to strengthen the interconnectivity of pulp material, resulting in increased mechanical strength.

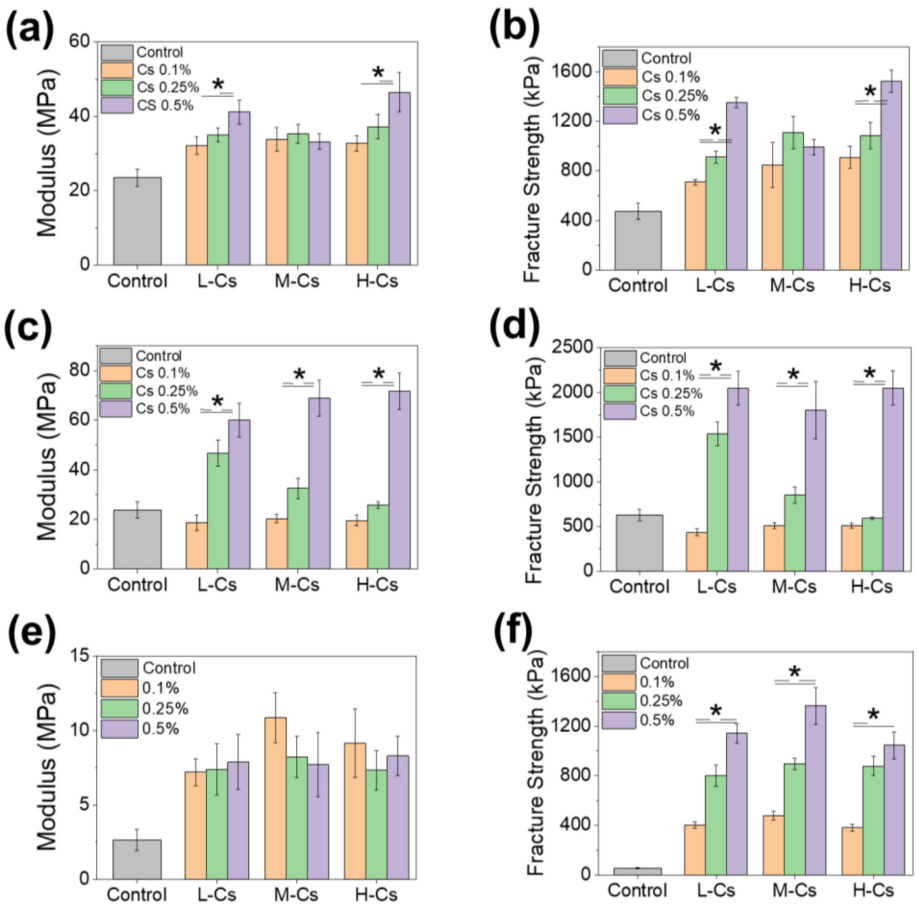

Figure 8. Tensile moduli and fracture strength of (**a**,**b**) Low S% seaweed paper, (**c**,**d**) High S% seaweed paper and (**e**,**f**) plant paper coated with chitosan with varying concentrations and molecular weights M_W (* $p < 0.05$). Uncoated papers were used as control.

As expected, the tensile mechanical properties, as identified by tensile moduli and ultimate strength, were shown to increase with the chitosan coating. It also resulted in a small but significant increase in elongation at break (Figure S5). The effects of chitosan concentration and M_W on the tensile mechanical properties generally coincided with the reduction in WAC, which was a clear indication that the chitosan coating itself was responsible for improving both water repellency and mechanical strength. The tensile moduli and fracture strengths of Low S% and High S% seaweed papers all increased with concentration and M_W of chitosan, but the degree of increase was more substantially pronounced for High S% over Low S% (Figure 8a–d). For example, the tensile moduli of uncoated Low S% and High S% seaweed papers were similar at 23.7 (±5) and 23.5 (±2) MPa, respectively. The highest tensile moduli of Low S% and High S% seaweed papers attained by 0.5% chitosan coating increased significantly up to 46.5 (±53) and 71.7 (±7.3) MPa, respectively, which were 2-fold and 3-fold increases from the uncoated samples (Figure 8a,c). Similarly, the fracture strengths of uncoated Low S% and High S% seaweed papers were also similar at 480 (±53) and 530 (±75) kPa, respectively. However, the highest fracture strengths were 1520 (±92) and 2050 (±190) kPa, respectively, which were 3.1-fold and 3.9-fold increases from the uncoated papers (Figure 8b,d). Overall, these results provided clear evidence that chitosan was more effectively coated onto seaweed paper with higher seaweed content and helped improve their mechanical strengths even though chitosan coating did benefit both types of papers.

The effect of chitosan coating on the mechanical strength of plant paper having critically low tensile strengths was further explored (Figure 8e–f, Figure S6). The tensile moduli ranged from 7.5 to 10.6 MPa by chitosan coating, which is a significant improvement from the uncoated paper with 2.5 MPa, although it was not greatly affected by M_W and concentration of chitosan. More notably, the chitosan coating resulted in fracture strengths up to 1360 (±150) kPa, which was a remarkable 25-fold increase from the uncoated paper with 54 (±4) kPa. Greater mechanical enhancement by chitosan coating for plant paper further highlighted the highly favorable physical interaction between chitosan and plant pulp, which contains high anionic polysaccharide contents.

3.4. Antimicrobial Effect of Chitosan-Coated Paper

Paper is increasingly used for food packaging, with non-degradable plastic waste causing serious environmental and health issues worldwide. It is, therefore, not only important for paper packaging to demonstrate moisture-resistance, but it must also be desirable with respect to antimicrobial effects for prolonged storage and protection. In this regard, using chitosan as a coating material could bring about another benefit due to its natural antimicrobial properties [24,26,27,40]. Thus, the antimicrobial properties of chitosan-coated paper were examined by measuring the growth of *E. coli* cultured on the paper via an MTT assay.

As expected, chitosan coated seaweed paper and plant paper demonstrated substantial antimicrobial activity, as *E*. coli growth was greatly inhibited with increasing chitosan concentration (Figure 9). This result clearly demonstrated that coating the papers with chitosan could impart significant antimicrobial activity for possible applications in the storage of highly sensitive materials.

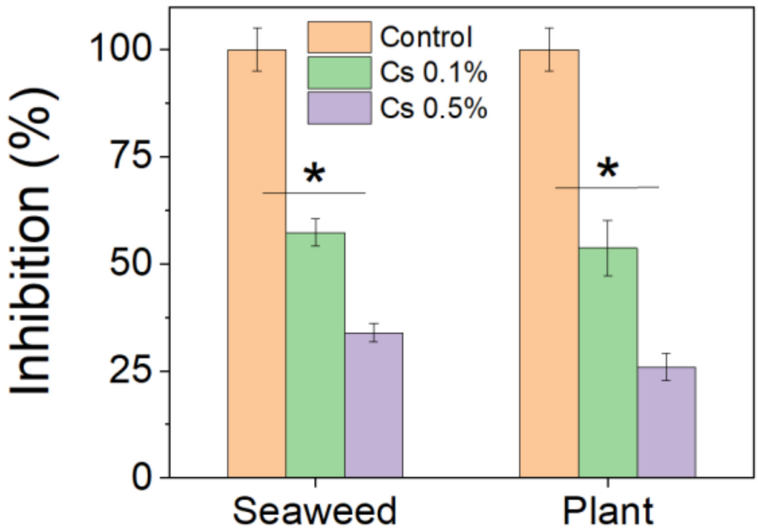

Figure 9. Antimicrobial effect of chitosan-coated paper was assessed by measuring the degree of inhibition (%) of *E. coli* growth on seaweed (High S%) paper and plant paper coated with chitosan (M-Cs) (* $p < 0.05$).

3.5. Surface Topography of Chitosan-Coated Paper

In order to examine detailed surface topographical features of chitosan-coated papers, optical coherence tomography (OCT) was utilized (Figure 10a). OCT allows non-destructive imaging of optical scattering media such as biological tissues and low density paper and plastics in high resolution and depth [41]. It was evident that uncoated paper showed high surface roughness, likely due to the presence of larger fibers of the pulp. With chitosan coatings, surface roughness decreased and became more level with increasing chitosan concentrations [42].

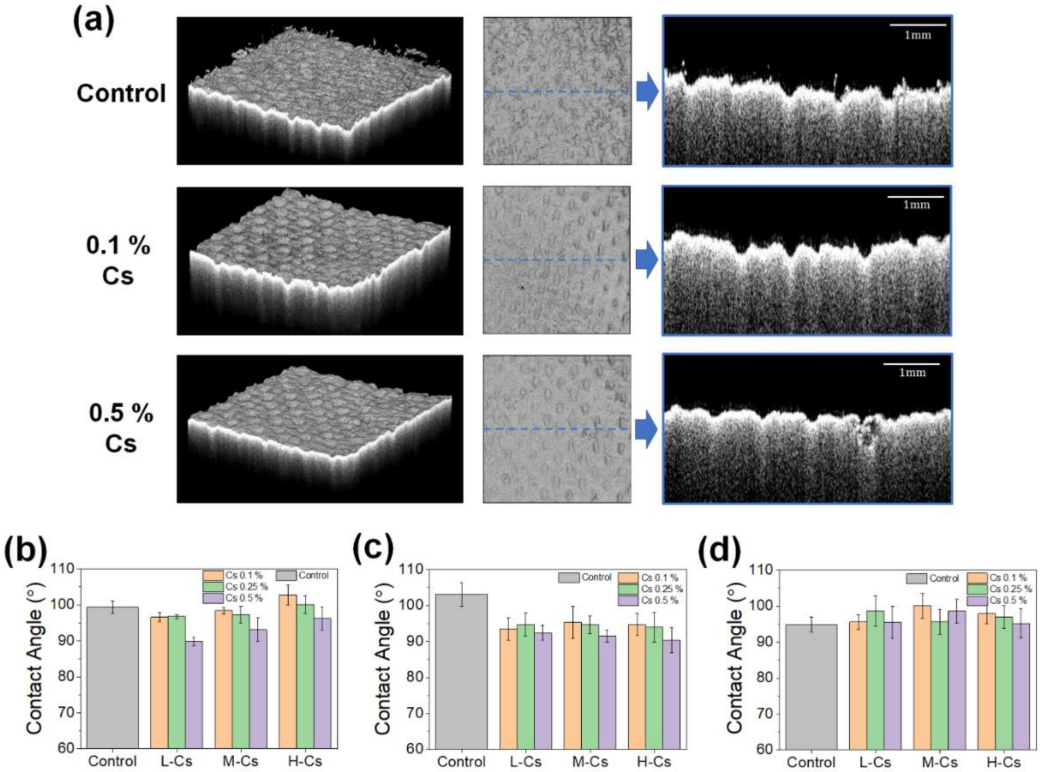

Figure 10. (**a**) OCT imaging of the surface of seaweed paper (High S%) coated with varying chitosan concentrations; 0% (control), 0.1% and 0.5%. Three-dimensional and cross-sectional (along the dotted line) views are presented. Water contact angles measured on chitosan-coated papers: (**b**) Low S% seaweed paper, (**c**) High S% seaweed paper and (**d**) plant paper. Uncoated papers were used as control.

The decrease in surface roughness was further manifested in water contact angles (Figure 10b–d). For Low S% seaweed paper, chitosan-coating at high concentrations of 0.5% resulted in a small decrease in contact angle despite showing significant water-repellent behavior (Figure 10b). For High S% seaweed papers, there was a similar decrease in contact angle regardless of chitosan concentration (Figure 10c). This suggested that the decrease in contact angle by chitosan coating was largely indicative of reduced surface roughness rather than hydrophobic effects, which was evident in the OCT images shown in Figure 8a. For plant paper, however, the contact angle was not significantly affected by chitosan coating (Figure 10d). This is likely a result of much greater increases in water repellent property and the resultant increase in contact angle, as compared with Low S% and High S% seaweed papers, offsetting the decrease in contact angle by reduced surface roughness. Regardless, these observations overall indicated that the consequence of chitosan coating was not only the deposition of chitosan that could resist aqueous dissolution but also eliminating rough edges resulting in smoother surface. Uneven and rough areas of the papers are defect sites that can result in mechanical failure and water absorption. By applying chitosan coatings, the surface defects could be significantly decreased, resulting in water repellency and enhanced mechanical strength.

4. Conclusions

Despite its immense potential as an eco-friendly alternative to traditional wood pulp-based paper, seaweed and non-wood plant-based papers suffer from diminished mechanical strength and vulnerability to moisture, which prevent more widespread usage. In order to overcome these issues, chitosan was used as a multifunctional coating material to impart water repellency and antimicrobial effect and to improve the mechanical strength of seaweed and plant papers. The effects of molecular weight and concentration of chitosan on the degree of water repellency and mechanical properties were systematically examined. It was clearly evident that the reinforcing effects of chitosan were more dominantly demonstrated for seaweed paper with higher seaweed content and plant paper. This is due to the greater degree of physical interaction between cationic chitosan and seaweed and plant pulps, which largely contain anionic polysaccharides via electrostatic interaction. The natural antimicrobial effect of chitosan was also effectively relayed to the chitosan-coated paper. Taken all together, chitosan coating provides an effective and highly practical approach to provide moisture-resistance and mechanical strength and antimicrobial effects relative to eco-friendly papers from regenerated natural sources.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/coatings11111384/s1, Figure S1: TNBS assay to determine amine content of chitosan-coated paper, Figure S2: A photograph and schematic illustration of spray nozzle used for spray coating, Figure S3: FT-IR spectra of uncoated and chitosan-coated seaweed paper and pure chitosan, Figure S4: Photographs of Low S% seaweed paper samples coated with chitosan after immersing them in hot water, Figure S5: Stress-strain curves of (a–c) Low S% and (d-f) High S% seaweed papers coated with chitosan with varying MW's and concentrations, Figure S6: Stress-strain curves of non-wood plant papers coated with chitosan with varying MW's and concentrations.

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